REMARKS

In this Amendment, claims 11-14 and 26-27 have been amended, and claims 28-34 are new. After entry of this Amendment, claims 11-14 and 25-33 will be pending in this Application.

The amended claims and new claims are supported by the specification as follows.

Amended claims 11, 13, 14, 26, and 27 recite a "a sterilized nutrient composition, wherein said composition is a biomass." This language is intended to clarify the original claim language, and finds support in the specification at page 2, second full paragraph, and the paragraph bridging pages 3 and 4.

Independent claim 11 has been amended to recite that the biomass "is generated from bacterial cells, wherein said bacterial cells comprise: (i) at least one species of methanotrophic bacteria and (ii) at least one species of heterotrophic bacteria." This amendment is supported by the specification at page 3, second full paragraph.

Independent claim 11 has also been amended to recite "at least sterile nutrient *added to the biomass*." This amendment is supported by the specification at page 3, second full paragraph, and the paragraph bridging pages 4 and 5.

New claims 28, 29, and 31 are supported by the specification at page 3, second full paragraph. Further, the bacterial strains recited in claims 28 and 29 were deposited with the NCIMB (The National Collection of Industrial, Marine and Food Bacteria) and are freely available to any third party on request.

New claims 30 and 34 are supported by the specification at the paragraph bridging pages 3 and 4.

New claims 32 and 33 are supported by the specification at page 2, fourth full paragraph.

The amendments to claims 12, 13, 14, 26, and 27 have been made for clarity and to be consistent with the independent claim.

No new matter has been added.

Entry of this Amendment is respectfully requested.

I. Response to Claim Rejections Under 35 USC §112, Second Paragraph

At page 2 of the Office Action, claims 13-14 and 26-27 are rejected under 35 USC §112, second paragraph, as being indefinite. Specifically, the Examiner states that the language "biomass deriving component" renders these claims indefinite.

The claims have been amended to refer to a "biomass."

Withdrawal of this rejection is requested.

II. Response to Claim Rejections Under 35 USC §102

At page 3 of the Office Action, claim 11-12 and 25 are rejected under 35 USC §102(b) as being anticipated by Patz et al. (DD 290,917).

Specifically, the Examiner states that Patz et al. teach a microorganism growth substrate comprising a chemical thermal hydrolysate derived from a culture of *Methylobacterium rhodesianum*, which the Examiner contends meets the limitation of "a sterile nutrient composition derived from the biomass of a culture of bacteria including methanotrophic bacteria," as recited in unamended claim 11.

The Examiner further states that the growth substrate of Patz comprises a sterile nutrient such as methanol and optionally contains a diluent such as water, as also recited in independent claim 11.

First, submitted with this Amendment is an English language translation of Patz et al., which Applicants believe to be a more accurate translation than that currently of record.

Amended independent claim 11 recites "a sterilized nutrient composition, wherein said composition is a biomass generated from bacterial cells, wherein said bacterial cells comprise: (i) ***at least one species of methanotrophic bacteria, and (ii) at least one species of heterotrophic bacteria***...."

Patz et al. do not disclose such a sterilized nutrient composition because Patz et al. do not disclose a biomass generated from bacterial cells comprising at least one species of methanotrophic bacteria[1] and at least one species of heterotrophic bacteria. Patz et al. merely disclose cultivating *Methylobacterium rhodesianum* where the nutrient source may comprise a hydrolysate of the bacteria itself (see page 4, lines 17-18 and claim 1 of the enclosed translation).

Thus, withdrawal of this rejection is requested.

III. Response to Rejection Under 35 USC §103(a)

(1) At page 5 of the Office Action, the Examiner rejects claims 11-12 and 25 under 35 USC §103(a) as being obvious over Patz et al., in view of Koffas et al. (U.S. Patent 6,689,601), and as supported by Arcangeli & Arvin.

[1] The term "methanotrophic" encompasses not only bacteria which grow on methane but also those which grow on methanol.

Specifically, the Examiner acknowledges that Patz does not disclose a sterile nutrient composition derived from a biomass obtained from the culture of methanotrophic bacteria.

However, the Examiner contends that Koffas teaches a high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate such as methane or methanol, and which is particularly suitable for the production of biomass.

The Examiner concludes that it would have been obvious to a person of ordinary skill in the art at the time the present invention was made to modify the growth substrate composition of Patz, by substituting the sterile nutrient composition derived from a biomass of methanotrophic bacteria taught by Koffas.

The Examiner further contends that a conclusion of obviousness is further supported by Arcangeli & Arvin, which allegedly teach that heterotrophs co-exist with methanotrophs in certain biofilms.

Amended independent claim 11 recites a sterilized nutrient composition, wherein said composition is a biomass generated from bacterial cells, wherein said bacterial cells comprise: (i) at least one species of methanotrophic bacteria, and (ii) at least one species of heterotrophic bacteria."

Neither Patz et al. nor Koffas teach such a sterilized nutrient composition as claimed.

Arcangeli & Arvin also fail to teach such a sterilized nutrient composition. While Arcangeli & Arvin may indicate that heterotrophs co-exist with methanotrophs in certain biofilms, Arcangeli & Arvin do not suggest that a biomass of such heterotrophs and methanotrophs would be a useful component in a microorganism growth medium. In other

words, Arcangeli & Arvin do not suggest that a biomass of heterotrophs and methanotrophs would be useful for any purpose, let alone that which is the subject of the present invention.

In contrast, the present Application shows that the growth media according to the present invention is effective for growing a broad spectrum of microorganisms, which therefore is advantageous for culturing unknown microorganisms. This benefit is not taught or suggested by the teachings of the prior art.

(2) At page 8 of the Office Action, claims 11-14 and 25-27 are rejected under 35 USC §103(a) as being obvious over Patz et al., in view of Koffas (as applied above), and further in view of Atlas & Parks, Handbook of Microbiological Media (1993).

Specifically, the Examiner acknowledges that a microorganism growth substrate comprising an additional sterile nutrient, such as glucose, nitrate and mineral salts, and in the amounts recited in claims 13, 14, 26 and 27, is not explicitly disclosed by Patz, Koffas, or Arcageli & Arvin.

However, the Examiner contends that Atlas & Parks teach various nutrient media compositions routinely used for the cultivation of bacteria, including sterile nutrients such as glucose, nitrate, and mineral salts.

Amended independent claim 11 recites "a sterilized nutrient composition, wherein said composition is a biomass generated from bacterial cells, wherein said bacterial cells comprise: (i) at least one species of methanotrophic bacteria, and (ii) at least one species of heterotrophic bacteria."

Neither Patz, Koffas, or Atlas & Parks, either alone or in combination, teach a sterilized nutrient composition as recited in independent claim 11. That is, like Patz and Koffas, Atlas & Parks also do not teach or suggest a biomass generated from bacterial cells comprising at least one species of methanotrophic bacteria and at least one species of heterotrophic bacteria.

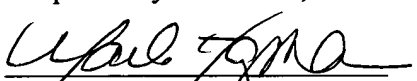
Accordingly, withdrawal of this rejection is requested.

IV. Conclusion

In view of the above, reconsideration of this application is requested. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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German Patent 290 917

Process for producing biogenic extracellular flocculating agents

Patent Claims:

1. Process for producing biogenic flocculating agents by cultivating bacteria using hydrolysed biomass as a nutrient source, characterised in that the new strain Methylobacterium rhodesianum IMET 11401 is cultivated with at least part of the growth substrate being substituted by chemical-thermal hydrolysate of bacterial biomass.

2. Process according to claim 1, characterised by the use of a hydrolysate biomass which is obtained at a pH>8 and in the temperature range from 80 to 170°C.

Scope of the application

The invention relates to the production of a bio-flocculating agent which can be used for example for concentrating sediment and particularly biomasses, preferably for purifying waster water.

Characterisation of the known technical solutions

Various methods are known for obtaining flocculating agents of microbial origin. Such flocculating agents are intended for various applications, e.g. for water treatment, for use for medical purposes, paper manufacture, etc. The microorganisms, fungi and bacteria used are wide-ranging in terms of their taxonomic classification and the compounds with a flocculating effect are either not specifically characterised in terms of their nature or are classed as

proteins, polysaccharides or fatty acids. In most known cases the production of bioflocculating agents gives rise to considerable problems. Thus, the cultivation process is generally accompanied by a low space-time yield, the clean substrates needed for growth and product formation are a significant cost factor in the process and expensive processing operations are required in order to obtain the product. Consequently, known methods are not an option for industrial application of the flocculating agent, e.g. in waste water purification.

A process is known in which bacteria of the genus Acetobacter methanolicus are cultivated and the secretion of biopolymers with a flocculating effect is induced by P limitation. This process is able to avoid some of the disadvantages of the prior art described above. However, the product-forming bacteria require a pH range for their cultivation which is detrimental to later use of the bioflocculating agent in waste water purification if the intention is to use not purified product but culture fluid containing product. Moreover, it is necessary to use methanol as a substrate for cultivating the bacteria claimed, which adds considerably to the cost. At least partial substitution of the substrate by enzymatically produced hydrolysed biomass as described in US 4,041,182 for cultivating mixed bacterial populations is impossible with these bacteria as they do not use any carbon sources other than glucose and methanol. Finally, the productivity of the product formation is not high enough to warrant industrial use of the process.

Aim of the invention

The invention sets out to produce microbial flocculating agents using a cheap substrate, in a high yield.

Essence of the invention

The invention is based on the task of providing and using microorganisms of this kind for producing extracellular bioflocculating agents which can be cultivated using hydrolysed biomass as a nutrient source in the neutral range.

According to the invention this objective is achieved by the fact that the new bacterial strain Methylobacterium rhodesianum IMET 11401 is cultivated with the growth substrate at least partially substituted by a chemical/ thermal hydrolysate of bacterial biomass. The new strain of bacteria was deposited at the culture collection of the Central Institute for Microbiology and Experimental Therapy in Jena in the GDR. The cultivation conditions are characterised by a pH range from 6.5 to 7.3 and a temperature range of 25 to 40°C. It has the following characteristics:

Cell morphology

gram-negative to gram-variable plump rods (0.8-1.2 x 1.5-4.0 µm), mobile, non spore-forming, cells often branched and pleomorphic, often containing inclusions of PHB

Colony morphology

pink colonies: round, glossy, smooth-edged, convex, greasy consistency, diameter <0, max 1mm (standard agar with methanol); peptone agar: reddish-orange colonies

Physiological features

Growth	Strictly aerobic
Oxidase	+

Catalase	+
NO_3 reduction	+
Urease	+
Methyl-red reaction	-
H_2 formation	-
Indol formation	-
Amylase	- (+)
Gelatinase	-
Lipase	-
Lecithinase	-
Lysine decarboxylase	-
Ornithin decarboxylase	-
Arginine dihydrolase	-
Need for growth factor	-

The hydrolysed biomass may be obtained both from the actual bacteria used and from other municipal or industrial biomasses provided that they do not contain any inhibitory substances which would affect bacterial growth. It is preferable to use activated sludge from municipal or industrial sewage treatment plants. The hydrolysed biomass is obtained from the above mentioned bacterial masses by treating at pH > 8 in a temperature range from 80 to 170°C. Preferably, brief heating takes place at 120°C at a pH of about 12. Depending on the starting materials, the hydrolysates contain lower amines, peptide fragments, mono- and oligosaccharides including amino sugars and a mixture of insoluble cell components. The latter may advantageously be removed before the hydrolysate is used. The advantage of thermal/chemical as against enzymatic hydrolysis is that the heavy metals contained in the biosludge are precipitated under these reaction conditions or are absorbed into the solid and can therefore no longer have a toxic effect on the substances forming the flocculating agents once the solids have been removed.

Moreover, total sterilisation of the biosludge and the use of basic industrial chemicals which are cheaper than enzymes and buffer substances is a major advantage of the process according to the invention.

The bacterial hydrolysate may partially or even completely replace the substrate methanol conventionally used for the strain of bacteria, while in some cases it may be necessary to adjust the equilibrium of the C/N ratio. Under otherwise identical optimum growth conditions the biopolymers are secreted without the usual need to limit a nutrient content. The extracellular biopolymers are water-soluble. They occur partly in aggregated form with a coil diameter of > 0.4 µm. The content of amino groups in the carbohydrate molecule fluctuates between 2 and 16%. With the process according to the invention it is possible to produce bioflocculating agents in the neutral range, using a cheap secondary substrate, which may preferably be used in sewage treatment, and wherein the culture medium obtained can be used without any further processing. The content of bioflocculating agents may be up to 0.4 g/g of BM and is thus significantly higher than in the processes known up till now. The bioflocculating agent may be obtained from the culture medium by conventional methods, e.g. by precipitation with isopropanol, filtrating and drying.

The invention is illustrated by the examples that follow.

Exemplified Embodiments

Example 1:

Bacteria of Methylobacterium rhodesianum IMET 11401 are fermented discontinuously at 32°C and at pH 6.2-6.8. Hydrolysed biosludge and methanol are used as the source

of carbon and nitrogen. The hydrolysed biosludge is obtained by brief heating to 150°C at a pH of 12 from secondary excess sludge with subsequent separation of the solids. The hydrolysate has a C:N ratio of about 4.6:1. As this ratio is insufficient for growth of the microorganisms or secretion of exopolysaccharides, methanol has to be added to achieve a balance (one part of hydrolysed activated sludge = 0.14 parts of methanol - 100% by volume). After 72 hours' cultivation the flocculating agents precipitated into the processing water by the microorganisms are separated off through a membrane cell using cross-current microfiltration. The suspension containing the microorganisms is circulated between a fermenter and the membrane cell. Once the mass of the fermenter content has fallen by 33% the removal of the flocculating agent is stopped and the content is topped up again with a substrate mixture consisting of methanol and hydrolysate.

The alternating processes of adding the substrate and removing the flocculating agent can be repeated as often as desired. The flocculating agent is precipitated from the processing water using isopropanol, filtered off and dried. The concentrations of flocculating agents achieved were about 250 mg of KH/l or 120 mg of KH/g BTS.

Example 2:

The bacterial culture IMET 11401 is fermented continuously over a period of from 8 to 10 hours. The inorganic nutrient solution has the following composition:

Compound	Requirement of element in mg/g BTS
KCl	10
H_3PO_4 (80%)	20

$MgSO_4 \times 7H_2O$	2.5
$CuSO_4 \times 5H_2O$	0.2
$FeSO_4 \times 7H_2O$	0.337
$ZnCl_2$	0.154
$MnSO_4$	0.464
$CoSO_4 \times 7H_2O$	0.0075
$NiCl_2 \times 6H_2O$	0.0228
$CrCl_3 \times 6H_2O$	0.015
$Al_2(SO_4)_3$	0.015
$Ca(NO_3)_2 \times 4H_2O$	0.1499
H_3BO_4	0.225
$Na_2MoO_4 \times 2H_2O$	0.0163

Methanol was used as the C source. The residual P concentration is controlled so that it is alternately limited or excessive. The fermentation suspension is pumped in an external circuit through a membrane which lets through the aqueous medium with the flocculating agent and holds back the biomass. The biomass is partially recycled and partially removed. The flocculating agent is recovered by precipitation, filtration and drying.

The concentrations of flocculating agent achieved were 650 mg of KH/l or 116.5 mg of KH/g BTS.